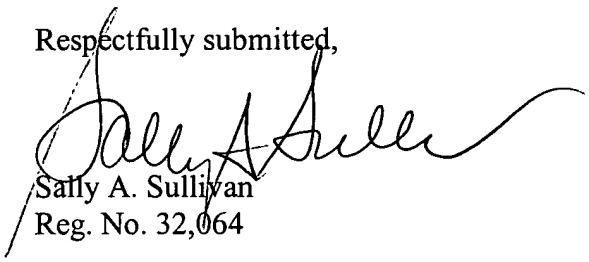
Applicants provide replacement sheets incorporating the amendments requested in the amendment herein. Because Applicant was required to submitted a replacement specification to correct improper margins in the specification as filed, the replacement sheets are not paginated as in the specification as filed. The undersigned states that the replacement pages submitted do not contain any changes except those recited above and do not contain and new matter. Replacement pages 22, 23, 43 and 78 are submitted.

Also submitted are Marked-up copies of the amended paragraphs and claims.

This submission does not require the payment of a fee. If this is incorrect, please deduct any required fee from deposit account 07/1969.

Respectfully submitted,



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MARKED UP COPY OF AMENDMENTS

In the Specification

Please amend the specification at page 18, lines 3-19 as follows:

Multivalent ligands of this invention can be used to modulate signal transduction in prokaryotic and eukaryotic organisms. The ligands function in a variety of signal transduction processes. Prokaryotes have a highly conserved intracellular signal transduction system, the two component system. The major components of this system are varying numbers of alternating histidine-aspartic acid kinase-mediated phosphorylation events, such as virulence, antibiotic resistance, response to environmental stress and sensing. The components of the two component system are highly conserved in prokaryotes. In contrast, eukaryotes appear to have very few two component systems for signal transduction. This orthogonality makes the two component signaling pathway a prime target for exploitation in therapeutic design for the control of bacterial infection. Major signal transduction systems in eukaryotes are mediated by G-protein-linked receptors and enzyme-linked receptors (including receptor guanylyl cyclases, receptor tyrosine kinases, tyrosine-kinase-associated receptors, receptor tyrosine phosophatases, and receptor serine/threonine kinases). The ability to modulate or regulate signal transduction in these pathways allows control over a wide variety of biological processes in eukaryotic cells and eukaryotic [organism] organisms (including mammals and specifically humans) and provides significant opportunity for the design of therapeutics.

Please amend the paragraph at page 22, lines 10-19 as follows:

RE is a recognition element as discussed above that can be any of a variety of chemical or biochemical species that are recognized by and which selectively bind to cell receptors, particularly, transmembrane receptors and cell surface receptors. SRE is a signal recognition element as discussed above that can be any of a variety of chemical or biochemical species that are recognized by one or more cells and which induce a

biological response by the cell; "L" is an optional linker group that can provide functional groups for covalent bonding of the RE, SRE or FE to the polymer (oligomer) backbone. FE is a chemical or biochemical functional group other than an SRE, as discussed above. Other examples of [ROM] ROMP scaffolds are illustrated in Schemes 2 and 3.

In the specification at pages 23-24, please amend the paragaraph bridging the pages as follows:

The linker can provide for spacing of the RE, SRE or FE group(s) from the backbone or can provide for structural flexibility. Linkers may be the same or different on different monomers in the polymer. Linkers that are used in a monomeric scaffold to bond to RE, SRE or FE can also be all the same or different. In a given multivalent ligand carrying one type of RE or SRE group, the linker is preferably the same throughout the polymer. Linkers are generally selected so that they are compatible with the intended application of the multivalent ligand and to avoid interference with the function of signal groups. The linker is preferably linear and preferably ranges in length from 1 to about 20 atoms. The linker may contain alicyclic groups (such as a cyclohexyl group). The linker can be an alkyl chain carrying functional groups for bonding to the backbone of the ligand and to the signal. The linker can also be an ether, ester, ketone, amine, amide or thioether chain. In a specific embodiment, the linker can be described as an linear alkyl chain having from 1 to about 20 carbon atoms in length in which one or more non-neighboring CH_2 groups are optionally replaced with an -O-, -S-, -NH-, -NR^{10}-, -CO-, -NH-CO- , -O-CO-, [-C=C-] -HC=CH-, or -C≡C- group, where R^{10} is an alkyl or aryl group. Linker CH_2 groups can be substituted with halogens, alkoxy, or alkyl groups. In the absence of a linker group, the ROMP backbone or the signal group itself must provide the functionality for covalent bonding of the signal to the backbone. Exemplary linkers include those illustrated in Scheme 3.

Please amend the specification at pages 44 and 45 to rewrite the paragraph bridging the pages as follows:

Further experiments were conducted which demonstrated that ConA-mediated agglutination of erythrocytes could be controlled by addition of multivalent ligands (compounds **9-13**). Certain combinations of ConA and multivalent ligands exhibited enhanced agglutination of these cells compared to ConA itself, as shown in Fig. 11. In particular, a combination of ConA tetramer and multivalent ligand (compound **13**) at concentration [ration] ratio 10:1 (based on tetrameric ConA and based on the number of mannose residues) exhibited significantly enhanced agglutination compared to ConA alone.

In the Claims

Please rewrite claim 100 as follows:

100. The multivalent ligand of claim 99 wherein the FE in the at least one L^2-FE group in the ligand is a [detectible] detectable label or a [reported] reporter group.

Figures 2B-E: Selected sample paths for bacteria (Gram Negative, *E. coli*) treated with buffer alone (B); 1 mM galactose (C); or 1mM compound **1** (D); or 1 mM compound **3** (E). Sample paths are derived from motion of representative bacteria from a treated bacterial population.

Figures 3A and 3B: Results of *E. coli* capillary accumulation assays. The number of bacteria accumulated is plotted versus the concentration of the attractant (galactose or compounds **1** -**4**, Scheme 1) calculated on a saccharide residue basis. (A): Results are shown for capillaries filled with buffer alone, compound **1**, and compound **2** or (B): buffer alone, compound **3** and compound **4** at the indicated concentrations. The vertical line at 1 mM indicates the concentration of maximum chemotaxis for the monomeric compound **1**. The concentrations used in this assay are not directly comparable to those used in the motion analysis experiments (see Figure 2A), because the gradient formed in the capillary assay is not defined. Results are the average of 3 to 6 experiments performed in duplicate and error bars represent a single standard deviation. Partial permeabilization was required to obtain chemotaxis towards **4**, and was utilized for all experiments [57].

Figure 4: Results of *B. subtilis* capillary accumulation assays using ROMP-derived glucose ligands (compound **5** -**7**, Scheme 1). Buffer alone, glucose, or glucose-bearing compounds **5**-**7** were used as attractants in the capillary accumulation assay. Results are shown for glucose, compound **5**, compound **6**, and compound **7**. Results are the average of at least four trials performed in duplicate and error bars represent single standard deviations.

Figures 5A and B: Results of video microscopy motion analysis experiments. (A): Bacteria (*E. coli)* were treated with increasing concentrations of serine (μM) after initial treatment (followed by a 2 min adaptation period) with buffer alone (■) or 10μM attractant: galactose (●), compound **1** (10mer, ▲) or compound **3** (25 mer,◆); (B) Bar graph of data for angular mean velocity taken from Fig. 6A at serine concentration 1μM. Initial treatment with compound **3** results in a significant enhancement of bacterial response to serine. Angular mean velocities varied approximately 14% between experiments performed on different days.

Figure 6: Multivalent ligands bind specifically to chemoreceptors and induce receptor reorganization. The illustration schematic represents fluorescently labeled **8** (10, 590 nm emission) bound to Trg (11) via GGBP(12). Trg is labeled with anti-Tsr antibody (13, 530 nm emission).

Figures 7A-D: Model of receptor reorganization by synthetic ligands. (A) Chemoreceptors are observed to form dimers (or multimers) (20) in the plasma membrane of

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noncovalently to the scaffold and can comprise a plurality of recognition elements (RE), or signal recognition elements (SRE), and can optionally comprise other functional elements (FE).

The RE, SRE and any FE can be bonded on to the molecular scaffold randomly or to a pre-selected pattern in which the arraignment of the RE, SRE and FE along the length of the scaffold matches a selected pattern, e.g., alternating different SRE or RE, selected spacing of different SRE or RE and the like.

The molecular scaffold can be rigid or flexible, hydrophilic or hydrophobic, symmetrical or unsymmetrical, have large surface area or small surface area, and interact or not with cell surface receptors. The molecular scaffold can be any of a variety of oligomers or polymers, including without limitation, polyacrylamides, polyesters, polyethers, polymethacrylates, polyols, and polyamino acids and corresponding oligomers. Molecular scaffolds can in general be linear polymers, branched polymers or cross-linked polymers. Preferred molecular scaffolds are biocompatible. Molecular scaffolds prepared by ROMP methods, as illustrated in several formulas herein, are preferred. Molecular scaffolds can be hydrophobic or can be made to be more hydrophilic by substitution with polar substituents, such as -OH. The scaffold can be substituted, in general, with any groups that do not interfere with signal activity and which provide desirable chemical and physical properties.

The term "recognition element" or RE is used herein to refer to chemical or biochemical species, groups or structures that function for binding to cell receptors and in particular function or binding to cell surface receptors. RE are bonded to molecular scaffolds in the multivalent ligands of this invention. An RE can be a ligand for a cell receptor or a portion of such a ligand that is functional for receptor binding and that has been modified to allow its bonding to a molecular scaffold. An RE can be chemically identical to a cell receptor ligand or it may be modified from the ligand as a result of or to facilitate bonding to the scaffold.

The term "signal recognition element" or SRE is used herein to refer to chemical or biochemical species, groups or structure that function as chemical or biochemical signals (see below) and that are bonded into multivalent ligands of this invention. The SRE is typically a signal (group or molecule) that has been modified to allow its bonding into the multivalent ligand. An SRE can be chemically identical to a signal or it may be modified from the signal as a result of or to facilitate bonding to the scaffold. The SRE is preferably bonded into the multivalent ligand such that the signal function of the group is minimally affected.

mammals and specifically humans) and provides significant opportunity for the design of therapeutics.

Figure 1 illustrates several mechanisms by which multivalent ligands of this invention can function as effectors of biological response. A multivalent ligand can be involved directly in signaling where SREs on the multivalent ligand bind to cell surface receptors, similar to monomeric ligands, and directly induce (or inhibit) a response. Use of a multivalent ligand of this invention with SRE attached to a molecular scaffold can facilitate receptor clustering or relocalization on the cell surface, localization of second messengers or simply generally increase the affinity by local increase in SRE (ligand) concentration. Multivalent ligands functioning through direct signaling can be employed in a variety of applications, including those based on disruption of biofilm formation or disruption of cell migration, are of particular interest for vaccines, and other therapeutics (cancer treatment and antibiotics).

Multivalent ligands of this invention can also be involved indirectly in signaling (see Fig. 1) affecting the response of a cell to another signal or ligand. Multivalent ligands may function to sensitize or prime cells for enhanced response to another ligand. Indirect signaling effects may be mediated by clustering or reorganization of one type of cell surface receptor which effectively results in the localization or reorganization of other types of cell surface receptors. Multivalent ligands functioning through indirect signaling can also be useful in a variety of applications, particularly those based on enhancement of a biological response, and are of particular interest for vaccines adjuvants and modulators of immune responses.

Multivalent ligands of this invention also have application simply in binding to or targeting of cells. A multivalent ligand containing at least one recognition element for binding to a cell surface receptor (RE) and containing a functional element (FE) targets the cell with that FE. If FE is a label or reporter group, the multivalent ligand acts to label the cell. If FE has a biological function, the multivalent ligand targets the cell with that function.

Multivalent ligands that contain a plurality of RE (SRE or both) can function in macromolecular assembly which need not involve any biological signaling function. In such applications, the multivalent ligand need not contain any SRE, the multivalent ligand need only contain more than one recognition element for binding to a cell surface receptor (a recognition element, RE) and preferably a plurality of REs. In such applications, the multivalent ligands directly or indirectly bind to more than one cell resulting in cell

biochemical species that are recognized by one or more cells and which induce a biological response by the cell; "L" is an optional linker group that can provide functional groups for covalent bonding of the RE, SRE or FE to the polymer (oligomer) backbone. FE is a chemical or biochemical functional group other than an SRE, as discussed above. Other examples of ROMP scaffolds are illustrated in Schemes 2 and 3.

The multivalent ligand of the above formula contains up to n RE, SRE or both. In specific embodiments all of the monomers carry an RE or SRE (the number of RE + SRE is n). In other specific embodiments, regions of spacer monomers that do not carry RE or SRE intervene between regions of monomers that carry RE or SRE. The RE and SRE attached to different monomers may be the same or different. In one embodiment, RE or SRE throughout the multivalent ligand are all the same. In another embodiment, the multivalent ligand contains more than one type of RE or SRE. In a specific embodiment, the multivalent ligand contains two different types of RE or SRE or an RE and an SRE. In this embodiment, the RE and SRE are non-randomly positioned in the ligand. Preferably monomers carrying the same RE or SRE are grouped into blocks (as in block polymers) within the multivalent ligand and spacer monomers are optionally positioned between blocks. In other embodiments, R^1 and R^2 together can form an RE or SRE.

RE and SRE are attached to the polymer (oligomer) backbone such that they substantially retain their function for binding cell receptors or as signals, respectively. For a given RE or SRE there may be several ways in which it can be bonded into the multivalent ligand, each of which may result in RE that are different in binding affinity or SRE that are different either in binding affinity or in the level or type of response induced. For example, a peptide signal may be bonding through its N-terminus, through its C-terminus or via an amino acid side group, such as through a lysine side group. The site of attachment of an RE or SRE to the multivalent ligand is preferably selected to minimize loss of binding function (RE) or to minimize loss of signal function (SRE) or alternatively the site of attachment may be selected to maximize signal function (SRE). An RE or SRE may nevertheless exhibit properties that are different from free ligands or free signals (e.g., the binding affinity of an SRE for a cell receptor may be different from that of free signal from which is was derived or which it mimics), but which do not destroy the function of an RE as a ligand or an SRE as a signal. RE can include a variety of known cell receptor ligands and in particular can include lectins. SRE can specifically include monosaccharides (e.g., glucose, galactose), disaccharides, polysaccharides (greater than 2 sugar residues), derivatized saccharides

(e.g.,acylated, sialated), peptides, derivatized peptides (e.g., N-formyl peptides), peptoids, various chemoattractants, and various epitopes. Note that a particular chemical or biological species may function as an RE with one type or kind of cell and as an SRE with another type or kind of cell.

The linker can provide for spacing of the RE, SRE or FE group(s) from the backbone or can provide for structural flexibility. Linkers may be the same or different on different monomers in the polymer. Linkers that are used in a monomeric scaffold to bond to RE, SRE or FE can also be all the same or different. In a given multivalent ligand carrying one type of RE or SRE group, the linker is preferably the same throughout the polymer. Linkers are generally selected so that they are compatible with the intended application of the multivalent ligand and to avoid interference with the function of signal groups. The linker is preferably linear and preferably ranges in length from 1 to about 20 atoms. The linker may contain alicyclic groups (such as a cyclohexyl group). The linker can be an alkyl chain carrying functional groups for bonding to the backbone of the ligand and to the signal. The linker can also be an ether, ester, ketone, amine, amide or thioether chain. In a specific embodiment, the linker can be described as an linear alkyl chain having from 1 to about 20 carbon atoms in length in which one or more non-neighboring CH_2 groups are optionally replaced with an -O-, -S-, -NH-, -NR^{10}-, -CO-, -NH-CO- , -O-CO-, -HC=CH- , or -C≡C- group, where R^{10} is an alkyl or aryl group. Linker CH_2 groups can be substituted with halogens, alkoxy, or alkyl groups. In the absence of a linker group, the ROMP backbone or the signal group itself must provide the functionality for covalent bonding of the signal to the backbone. Exemplary linkers include those illustrated in Scheme 3.

R^1, R^2, R^4, R^5, R^6, R^7, R^8 and R^9 can be organic groups. Organic groups include without limitation alkyl groups, alkenyl groups, and aryl groups as well as substituted alkyl, alkenyl and aryl groups. Substituents for alkyl, alkenyl and aryl groups include halogens (F, Cl, Br, I), -CN, -NO_2, -OH, -SH, -NH_2, -$N(R^{10})_2$, -SR^{10} and -OR^{10} where R^{10} is an alkyl or aryl group. Aryl groups may also contain alkyl or alkenyl substituents. Organic groups will typically have from 1 to about 20 carbon atoms, and preferably have 1 to about 10 carbon atoms. Alkyl groups may be straight-chain, branched or cyclic (or contain portions that are cyclic). One or more non-neighboring -CH_2- groups in an alkyl or alkenyl group can be replaced with -O-, -S- , -NH- or -NR^{10}, where R^{10} is an alkyl or aryl group.

R^6 and R^7 can be end-groups, such as those described in U.S. patent application 09/336,121 filed June 17, 1999 which is incorporated in its entirety herein for description of

fluorescence quenching was dependent not only on scaffold valency, but also on ligand concentration. Quenching first increased as scaffold concentration increased and then decreased again as the concentration was increased further. The absence of quenching at high scaffold (multivalent ligand) concentrations indicates that Con A clusters are disfavored at these concentrations, likely because of site saturation. The high concentration of scaffold compared to Con A favors occupation of each ligand binding site on Con A by individual polymers precluding clustering of multiple lectins.

The ability of Con A clusters formed on ROMP-derived polymers to aggregate Jurkat cells was examined initially by light microscopy (see Fig. 10). Con A alone was able to induce some Jurkat cell aggregation even at low concentrations (5 μg/mL). When monovalent Con A ligands such as methyl α-D-mannopyranoside or **9** were premixed with Con A they inhibited aggregation, presumably by destabilizing Con A - cell interactions. For Jurkat cells, inhibition occurred even at low concentrations (0.5 μM) of monovalent ligands. Interestingly, multivalent compounds **10-12** did not inhibit Jurkat cell aggregation at 0.5 μM, a concentration shown to be optimum for Con A cluster formation under similar conditions. Increasing the concentration of the multivalent ligand 10-fold (5 μM) abolished aggregation activity, consistent with site saturation. Thus it is possible to alternatively inhibit or promote cell surface-lectin interactions by varying scaffold valency and multivalent ligand concentration. The ability of Con A complexed to multivalent ligands to interact with cell surfaces was thus tunable.

Further experiments were conducted which demonstrated that ConA-mediated agglutination of erythrocytes could be controlled by addition of multivalent ligands (compounds **9-13**). Certain combinations of ConA and multivalent ligands exhibited enhanced agglutination of these cells compared to ConA itself, as shown in Fig. 11. In particular, a combination of ConA tetramer and multivalent ligand (compound **13**) at concentration ratio 10:1 (based on tetrameric ConA and based on the number of mannose residues) exhibited significantly enhanced agglutination compared to ConA alone.

Complexes containing multiple Con A tetramers were assembled readily on compounds **10-13** when intermediate multivalent ligand concentrations were used, but were not detectable when the concentration of the scaffold was either too low or too high. The concentration range over which such complexes are formed depends upon the relative concentrations of ConA and multivalent ligand (based on the number of ligands, RE or SRE) and upon the valency of multivalent ligand. This is generally true for any complex of a

each R^1 and R^2, independent of other R^1 and R^2 in the ligand, can be H, an organic group, a signal recognition element -L^1-SRE, a recognition element -L^2-RE or a functional element -L^3-FE, wherein at least one of the R^1 and R^2 groups in the ligand is -L^3-SRE;

wherein L^{1-3} represent optional linker groups;

R^4 and R^5 are H, an organic group or an end-group; and

R^6 and R^7 are H, an organic group or an end-group.

97. The multivalent ligand of claim 96 wherein one of the R^1 or R^2 groups in each repeating unit of the ligand is -L^1-SRE.

98. The multivalent ligand of claim 96 wherein at least one of the R^1 or R^2 groups in the ligand is -L^2-RE .

99. The multivalent ligand of claim 96 wherein at least one of the R^1 or R^2 groups in the ligand is -L^2-FE .

100. The multivalent ligand of claim 99 wherein the FE in the at least one -L^2-FE group in the ligand is a detectable label or a reporter group.

101. The multivalent ligand of claim 99 wherein the FE in the at least one -L^2-FE group in the ligand is an enzyme.

102. The multivalent ligand of claim 96 wherein at least one of SRE is a peptide or a derivatized peptide.

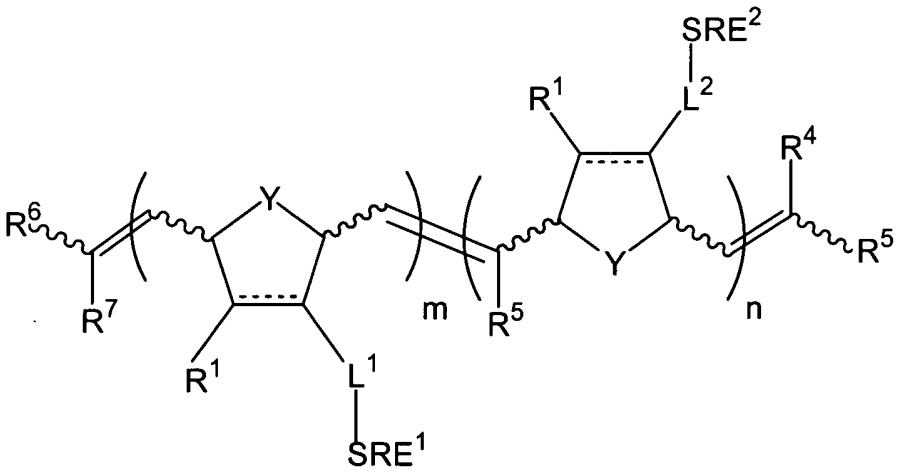
103. The multivalent ligand of claim 102 wherein at least one of SRE is an N-formyl peptide.

104. The multivalent ligand of claim 96 wherein at least one of SRE is an epitope.

105. The multivalent ligand of claim 104 wherein at least one of SRE binds to a cell surface receptor of an immune cell.

106. The multivalent ligand of claim 104 wherein at least one of SRE binds to a cell surface receptor of a B cell or a T cell.

107. The multivalent ligand of claim 96 having the structure:



wherein:

m + n is an integer of 2 or more and each integer represents the number of repeating units in the parentheses;

each Y, independent of other Y in the ligand, is -O-, -S-, -NR^8-, or -CH_2-;

R^1 can be H, an organic group, a -L^2-RE group or an -L^3-FE group;

L^1 and L^2, which may be the same or different, represent optional linker groups;

SRE^1 and SRE^2 represent two different signal recognition elements;

R^4 and R^5 are H, an organic group or an end-group; and

R^6 and R^7 are H, an organic group or an end-group.

108. The multivalent ligand of claim 107 wherein one of SRE^1 or SRE^2 is a peptide or a derivatized peptide and the other of SRE^1 or SRE^2 is a saccharide.

109. The multivalent ligand of claim 108 wherein SRE^1 or SRE^2 are two different peptides or derivatized peptides.